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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 2: B01D 13/00; A61M 1/03

A1

(11) International Publication Number: WO 80/02805

(43) International Publication Date: 24 December 1980 (24.12.80)

(21) International Application Number: PCT/SE79/00132

(22) International Filing Date: 11 June 1979 (11.06.79)

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(81) Designated States: AT (European patent), CH (European patent), DE (European patent), DE (Utility model), FR (European patent), GB (European patent), JP, LU (European patent), SE (European patent), US.

(82) Elected States: AT (European patent), DE (European patent), GB (European patent), JP, SE (European patent).

Published

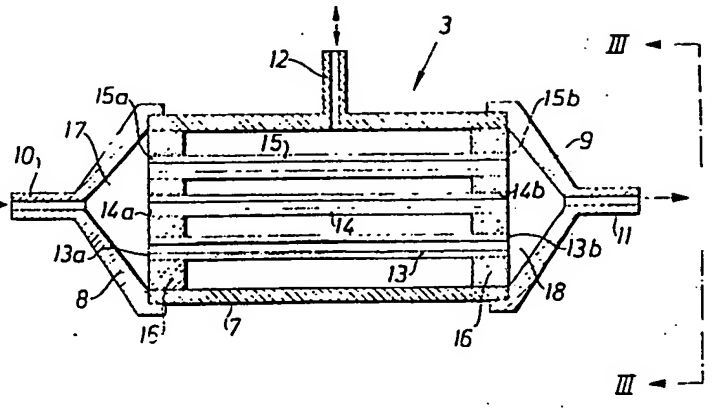
With international search report

In English translation (filed in Swedish)

(54) Title: PROCESS FOR THE TREATING OF AND/OR REMOVAL OF SUBSTANCES FROM A LIQUID, ESPECIALLY WHOLE BLOOD, AND A DEVICE AND A MEMBRANE FOR THE REALIZATION OF SAID PROCESS

(57) Abstract

A process for the treating of and/or removal of substances from a liquid, especially whole blood, via a semipermeable microporous membrane (13, 14, 15) through adsorption and/or biological reaction by means of a biologically active material. Said whole blood is exposed to pressure variations at the membrane surface in a way such that a penetrating fraction of said whole blood is flowing in an alternating flow path through the membrane walls for contacting said biologically active material. Said biologically active material is asymmetrically immobilized in and on the surface of the side of said membrane that faces away from said whole blood. Alternatively, said biologically active material may be bound to an unsoluble matrix behind said membrane. Said membrane is for example provided within a casing (7, 8, 9) comprising inlet (10) and outlet (11) for said whole blood. The space between said casing and said membrane is via a connecting nipple (12) in communication with for example an expansion chamber for the provision of pressure variations. By means of the device (3) it is possible to perform said process in one and the same step and by means of one and the same pumping system. Example of suitable materials for said membrane are regenerated cellulose, cellulose acetate, non-woven acrylic copolymer, polysulphone, polyether sulphone, polyacrylonitrile, polyamide and the like. The pores of said membrane are usually of the order of magnitude of 0.01 - 0.8 microns, preferably 0.15 - 0.45 microns. The choice of biologically active material is determined primarily by the type of substance that is to be removed.



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TITLE

PROCESS FOR THE TREATING OF AND/OR REMOVAL OF SUBSTANCES FROM A LIQUID, ESPECIALLY WHOLE BLOOD, AND A DEVICE AND A MEMBRANE FOR THE REALIZATION OF SAID PROCESS

TECHNICAL FIELD

This invention relates to a process for the treating of and/or removal of substances from a liquid via a microporous, semipermeable membrane through adsorption and/or biological reaction by means of a biologically active material. Furthermore, this invention relates to a device and a membrane for the realization of said process, and a process for immobilizing of a biologically active material on and/or in such a membrane.

Especially this invention is concerned with a process for the removal of substances from whole blood.

Example of such substances are immune complexes of IgG- or IgA-type having molecular sizes within the range of from $20x10^4$ to 10^6 Daltons. Further examples are antibodies, for example immunoglobulins of IgG-, IgM- and IgA-types, and antigens, for example virus and DNA in systemic lupus erytematosis (SLE). Also harmful enzymes, for example some proteolytic enzymes which are detrimental to human organs, are examples of such substances in this especial context.

Even though the present invention will be described with particular reference to a process for the treating of and/or removal of substances from whole blood, so-called blood immunotherapy, it is to be understood that the invention is not restricted to only this particular field of use. In its broader sense the present invention may be applied to other liquids which are to be treated in the way specified in the preamble of claim 1.

BACKGROUND OF INVENTION

Substances, for example macromolecules, in whole blood are usually captured or transformed specifically.

For example it is required that the blood corpuscles are initially separated from the plasma which is then treated separately for the removal of said macromolecules. Then, said plasma is filtered in further separate steps to remove possible remaining harmful substances, whereafter said plasma is finally rejoined with said blood corpuscles and fed back to the source for said whole blood.

If a rapid process is desirable, said separation is performed through plasmapheresis based on a centrifuge step. A more simple process is however realized, if said separation of whole blood is performed by means of a microporous, semipermeable membrane filter.

Irrespective if said separation of whole blood is realized through centrifuging or by means of a microporous, semipermeable membrane filter, two separate pumping systems are required in this known technique for the realization of said process, i.e. a pumping system for said whole blood and a pumping system for said plasma. The reason for this inconvenience is primarily that said process necessarily must be divided into more separate steps, as described hereinabove.

An object of the present invention thus is therefore to avoid said inconvenience in the known technique and to provide a process which does not require a dividing into separate steps for the treating of and/or removal of substances from the liquid, especially whole blood, and which therefore does not require two pumping systems.

Another object is to provide a device and a membrane for the realization of said process.

A further object is to provide a process for the immobilizing of biologically active material on and/or in such a membrane.

Other objects and advantages of the present invention will be apparent from the following description.

DESCRIPTION OF INVENTION

According to the present invention there is provided

a process for the treating of and/or removal of substances from a liquid, especially whole blood, via a microporous, semipermeable membrane through adsorption and/or biological reaction by means of a biologically active material. Said process is characterized in that a penetrating fraction of said liquid is forced to flow in an alternating path through said microporous membrane in each direction for contacting said biologically active material.

According to the present invention there is furthermore provided a device for the treating of and/or removal of substances from a liquid, especially whole blood, via a microporous semipermeable membrane through adsorption and/or biological reaction by means of a biologically active material. Said device is characterized in comprising means for the realization of an alternating flow of a penetrating fraction of said liquid through said membrane in each direction.

Furthermore, there is provided a microporous semipermeable membrane for the treating of and/or removal of substances from a liquid, especially whole blood, through adsorption and/or biological reaction by means of a biologically active material. Said membrane is characterized in that said biologically active material is immobilized in the pores and/or on the surface of the side of said membrane which is adapted to be faced away from said liquid.

Furthermore, there is provided a process for the immobilizing of IgG (biologically active material) on and/or in a microporous semipermeable membrane of amine substituted polyamide via glutardialdehyde. Said process is characterized in that one surface of said membrane is treated with a solution of glutardialdehyde in phosphate buffer; the so treated membrane is rinsed with distilled water and then sucked relatively dry; that IgG, dissolved in phosphate buffer, is poured over said membrane to cover same; that the system is evacuated; that the coupling is allowed to proceed; and in that said membrane finally is carefully rinsed with water.

Furthermore, there is provided a process for the

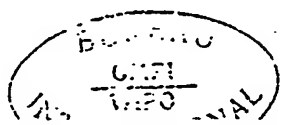
immobilizing of protein A (biologically active material) on and/or in a microporous, semipermeable membrane of cellulose acetate. Said process is characterized in that one surface of said membrane is treated with chloroacetic acid for coupling of carboxymethyl groups to said membrane; that said membrane is dried and then transferred into a degassed solution of phosphate buffer containing 1-ethyl-3(3-dimethylaminopropyl)carbodiimide; that the reaction is allowed to proceed, whereafter said membrane is washed with phosphate buffer to removing excess of 1-ethyl-3(3-dimethylaminopropyl) carbodiimide; that protein A is provided to cover said membrane which then is reacted with said protein; and in that said membrane finally is washed with water.

In the particular case where said liquid is constituted by whole blood, said biologically active material may be chosen from the group consisting of the following proteinaceous material: antibodies, antigens, enzymes, protein A and the like, for example killed bacteria or fragment thereof.

The choice of proteinaceous material is determined primarily of the type of substance that is to be treated and/or removed.

For example, if antibodies or complexes of antibodies and antigens, so-called immune complexes, are to be removed, protein A is preferably chosen, which preferably captures immune complexes of IgG-type, IgG and some IgM and IgA. Alternatively certain membrane protein from streptococci may be chosen, which relatively specifically binds to IgA and immune complexes thereof. A further alternative may be specific antigen in order to eliminate antibodies which are harmful to tissue, for example in transplantations.

If antigens, for example a virus or DNA in SLE, are to be removed, then it is convenient to choose an antibody which specifically binds to such antigens. Also antibodies or other fix adsorbents may be chosen to eliminate harmful enzymes, for example certain proteolytic enzymes which are detrimental to human organs.



Enzymes which perform biological reactions which the body does not manage to handle owing to any enzyme deficiency disease, constitutes examples of further biological material which can be used according to this invention.

The membrane according to the present invention may consist of any suitable blood compatible material which is able to bind to the biologically active material. Examples of such suitable material are regenerated cellulose, cellulose acetate, non-woven acrylic copolymer, polysulphon, polyethersulphon, polyacrylonitrile, polyamide and the like. The pores of said membrane are usually of the magnitude of order of 0.01 to 0.8 microns, preferably 0.15 to 0.45 microns.

Said membrane may be either in planar form or in form of one or more hollow fibers. In said planar form said membrane has the biologically active material bound in the pores and/or on the surface of the side of said membrane that faces away from the whole blood. When the membrane is in the form of hollow fibers, said biologically active material preferably is bound in the pores and/or on the surface of the outer side of said fiber or fibers. The whole blood is in this case adapted to flow through the longitudinal void in said fibers.

Alternatively, said membrane may be composed of two membrane halves which are mechanically generally identic to each other but which chemically may be built up of different material. In this case it is enough if only the membrane half that faces away from the whole blood is able to bind to the biologically active material. For example, said membrane halves may be provided in an abutting relationship to each other, wherein the biologically active material preferably is bound in the pores and on both surfaces of the membrane half that faces away from the whole blood.

Irrespective of the outer shape or chemical or mechanical constitution of said membrane, said biologically active material (for example protein A, antibodies, antigens, enzymes) must be immobilized in said membrane in such a way that the surface of said membrane that faces towards the whole blood is free of "reagent". This is to avoid contact

between blood corpuscles and the reagent and thereby pyrogen and/or anaphylactic reactions. Thus it is a form of asymmetric immobilization, where on one surface of said membrane as well as in the pores thereof said biological active material has been immobilized. The advantage of immobilizing within the pores on said membrane is that the active microscopic surface may be manifolded (>1000) compared to the macroscopic surface.

Through the asymmetric immobilizing of said biologically active material the diffusion distance from the blood to the biologically active material is very short (≤0.30 mm; thickness of membrane). Grace to this short distance a somewhat oscillating pressure wave is enough for providing desired transport of high molecular substances from the blood to said biologically active material.

Alternatively, said biologically active material may be bound to an unsoluble matrix behind said membrane. The treating process is yet similar, but since the necessary diffusion distance is about 10 times longer, it may be necessary to arrange a somewhat more real flow through said membrane.

Irrespective of whether the biological material is immobilized in the pores or an unsoluble matrix behind said membrane is used for immobilizing said active material, the immobilizing procedure must be so performed that said active material cannot be broken away. This means that covalent coupling is the most safe immobilization. What exact type of covalent coupling that is to be used depends on the choice of membrane material and the type of used biologically active material.

According to a preferred embodiment of the present device the microporous semipermeable membrane is used in the form of flat foils. Said foils are provided in pairs between distance plates and together with said distance plates

clamped within a casing comprising inlet and outlet for the whole blood. This construction differs from a so-called "plate kidney", which is known to the person skilled in the field of dialysis, primarily through the lack of corresponding inlet and outlet for dialysis solution. A more detailed description of this construction is therefore hardly necessary in this context. The whole blood to be released from substances, is pumped from for instance a patient into said casing through said inlet and is distributed in a convenient way through the spaces between said membrane foils being provided in pairs. During the passage through said spaces said whole blood is exposed to pressure variations in a manner so that only a penetrating fraction of said whole blood is caused to flow in an alternating path through the respective membrane foil in each direction for contacting said immobilized biologically active material. In this way said whole blood will be partly separated, partly treated within one and the same casing in one and the same step. Since the biologically active material is immobilized in the part of membrane that faces away from said whole blood said whole blood will not come into contact with said material. Consequently, any following separate filtering of said whole blood therefore is not necessary.

The means for the realization of said pressure variations may for instance be made up of an expansion chamber in fluid communication with the spaces between said membrane pairs and distance plates, respectively, which alternatingly is exposed to overpressure and underpressure in relation to the pressure of said whole blood.

According to another preferred embodiment of the present device said microporous semipermeable membrane is in the form of individual fibers which may be provided into bundles and encapsulated within one and the same casing comprising inlet and outlet for said whole blood. The ends of said fibers are glued by means of a suitable binder in a way such that said individual fibers are retained essentially parallel within said casing. One end of said fibers or

bundles of fibers is provided in communication with said inlet, while the opposite end is provided in communication with said outlet. Said whole blood is pumped into said casing through said inlet and through the longitudinal void of said fibers and out of said casing through said outlet. During said passage through said casing said whole blood, as described above, is exposed to said pressure variations, such that only a penetrating fraction of said whole blood is caused to flow in an alternating path through the fiber walls in each direction for contacting with said biologically active material. The means for the realization of said pressure variations may again be made up of an expansion chamber in communication with the space between said individual fibers and bundles of fibers, respectively. Again, the separation of whole blood as well as the capturing of said substances on the biologically active material is provided in a single step. Any following filtering of said whole blood for the removal of possible harmful residues is neither requested, since said filtering is automatically achieved through the passage of said plasma through said fiber walls.

Said pressure variations may vary from -200 to +200 mmHg, preferably from -100 to +100 mmHg. The longer the diffusion distance for the blood, for example if the biologically active material is bound to an unsoluble matrix behind said membrane, the higher compensating pressure variations are required to achieve the desired separation effect. In a corresponding way the frequency of said pressure variations may vary from about 0.05 up to about 10 Hz, preferably 0.5 to 1 Hz.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be described with reference to preferred embodiments of as well the process, the device as the microporous semipermeable membrane.

Fig. 1 is a schematic flow diagram showing the mode of carrying out the present process,

Fig. 2 is a longitudinal cross-section through a preferred embodiment of the present device, including microporous, semipermeable membranes in the form of fibers which are encapsulated within one and the same casing comprising inlet and outlet for the whole blood,

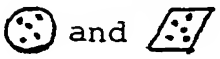
Fig. 3 is an end view of the device of Fig. 2,

Fig. 4 is part of a longitudinal cross-section through another preferred embodiment of the present device, wherein said microporous semipermeable membrane is in the form of individual fibers or thin-walled tubes, and

Figs. 5-6 show preferred embodiments of the microporous, semipermeable membrane according to the present invention, comprising immobilized biologically active material.

In Figs. 5-7 the following symbols have been used:

• = biologically active material

 and , respectively = granules comprising biologically active material.

THE MOST PREFERRED EMBODIMENT OF THE INVENTION

In order to remove for example antibodies or complexes of antibodies and antigens, so-called immune complexes, from a liquid, especially whole blood, said whole blood is withdrawn from a source 1 (Fig. 1), for example a patient, by means of a pump 2. Said pump 2, which may be a conventional blood pump, pumps said whole blood into the present device, generally designated 3. In said device said whole blood is exposed to pressure variations of the magnitude of order of from -200 to +200 mmHg, preferably from -100 to +100 mmHg, by means of an oscillation unit 4 which provides said pressure variations with a frequency of 0.05 - 10 Hz, preferably 0.5 - 1 Hz. In this way a penetrating fraction of said whole blood, i.e. the plasma, will flow in an alternating path through the walls of semipermeable microporous membranes in each direction, which are provided in the device 1, for contacting said biologically active material,

for example protein A. For more detailed information as regards said membranes reference is made to the following description. Said whole blood, which has passed through the device 3, is then pumped back into said patient 1 via a manometer 5 and an air detector 6. As well said manometer 5 as said air detector 6 are conventional in the field and need therefore not be described more in detail. The addition of anticoagulantia, as shown at 25, such as heparin, may be a pump or similar construction which in a well-known manner adds said heparin.

According to a preferred embodiment of the present invention the device 3, as shown in Figs. 2 and 3, consists of an essentially cylindrical intermediate part 7, both ends of which are closed by means of two identic conically shaped lids 8 and 9, respectively, while forming inlet 10 and outlet 11, respectively. Said intermediate part 7 is provided with a connecting nipple 12 for communication with said oscillation unit 4, as schematically shown by means of the two-headed arrow in Fig. 2.

The device according to Fig. 2 comprises microporous semipermeable membranes in the form of individual fibers 13-15, preferably of cellulose acetate. The ends of said fibers are glued by means of a suitable binder 16 in a manner such that said individual fibers 13-15 are essentially parallel within said casing. One end 13a, 14a, 15a of said fibers is provided in communication with said inlet 10, while the opposite ends 13b, 14b, 15b are provided in communication with said outlet 11. Said whole blood is pumped into the device 3 through said inlet 10 and is distributed into the space 17 between the fiber ends 13a-15a and the conical lid 8, such that essentially uniform portions of the entering whole blood will pass through the longitudinal void of each of said fibers 13-15. After the passage through said fibers said whole blood is collected in the space 18 between the fiber ends 13b-15b and the conical lid 9 and flows in a concentrated flow out of the device 3 through said outlet 11.

Even if only three individual fibers 13-15 are shown in

Fig. 2, said number may vary. For example said individual fibers may be provided in the form of bundles or bunches.

The embodiment shown in Fig. 4 differs from that of Figs. 2 and 3 primarily in that the microporous semipermeable membrane is constituted by one individual fiber 19. To support said fiber 19 the device comprises heads or concentric flanges 20 which are adapted to abut against the outer wall of said fiber.

In Figs. 5-7 different principles of immobilizing of said biologically active material are shown. In Fig. 5 said material is immobilized in and on the outer surfaces of flat membranes 21, 21'. In Fig. 6 the membrane 22 consists of two separate membrane halves 22a, 22b in an abutting relationship to each other. The biologically active material is immobilized on and in only the outer membrane half 22a. In Fig. 7 the biologically active material is received in granules 23 which are freely floating in the space around said microporous semipermeable membrane 24.

The following examples will illustrate concrete processes for coupling of said biologically active material to the present membrane.

EXAMPLE 1

In this example immobilizing of IgG to a polyamide membrane (nylon) is realized.

Amine substituted nylon membrane is manufactured as follows:

1. Said polyamide membrane is washed with 10% solution of Duponol RA (detergent) and is rinsed with distilled water and dried.

2. Triethyloxoniumtetrafluoroborate in dichloromethane was sucked into an evacuated vessel, wherein the membrane was applicated.

3. After 60 seconds the reagent solution was sucked off.

4. The membrane was rinsed through the so-called suction process with two portions of dichloromethane.

5. 1,6-diaminohexane was sucked into said reaction vessel and reacted with the activated groups of said membrane during 30 minutes at room temperature.

6. Finally, the membrane was flushed with distilled water during 24 hours and dried.

Coupling of IgG to the so treated membrane

In this example IgG was immobilized on and in the so treated membrane via glutardialdehyde. The membrane was therefore treated with 2.5% solution of glutardialdehyde in 0.1 M phosphate buffer at pH = 6.8. Then the membrane was amply rinsed with distilled water and sucked dry.

IgG, dissolved in 0.1 mM phosphate buffer (4°C) at pH = 6.0 was poured over the membrane, so that this was covered. The system was evacuated during 30 minutes, whereafter the coupling was allowed to proceed during further 15 hours at 4°C.

Not coupled IgG was collected with the first rinsing water in an ion exchanger. The membrane was carefully rinsed with water, whereafter it was ready to be applicated in the present device.

EXAMPLE 2

In this example protein A was immobilized on a cellulose membrane.

In a first step carboxymethyl groups were coupled to a membrane washed with an alcohol. This was realized in that the membrane material was treated with chloroacetic acid.

The membrane was dried, while being careful so as to avoid formation of cracks. 0.2 M phosphate buffer at pH 4.75 was degassed. 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (EDC) was added, such that the alcoholic concentration of this substance was 0.1 M. The membrane was then transferred into said degassed EDC solution. The reaction was allowed to proceed during 30 minutes at room temperature. Warm phosphate buffer of pH = 7.0 was used to wash said membrane free of excess of EDC.

Protein A in a concentration of 2 mg/ml was caused to cover said membrane. The membrane was then allowed to react with said protein during 24 hours at 4°C. Finally, the membrane was washed and was then ready to be applied in the present device.

INDUSTRIAL APPLICABILITY

The present process is especially, though not exclusively, useable for the treating of and/or removal of substances, such as enzymes, antigens and/or antibodies from whole blood. Said whole blood is pumped from for example a patient into a treating unit comprising a microporous semipermeable membrane having pores of 0.01 - 0.8 microns, preferably 0.15 - 0.45 microns. During the passage through the treating unit said whole blood is exposed to pressure variations (for example from -200 to +200 mmHg, preferably from -100 to +100 mmHg), whereby a penetrating fraction of said whole blood, i.e. the plasma, is caused to flow in an alternating path through the membrane wall in each direction for contacting the biologically active material.

Said biological active material may for example be antibodies, antigens, enzymes, protein A etc., for example killed bacteria or fragments thereof. The choice of biologically active material is determined primarily by the type of substances that are to be removed.

Suitable materials for the membrane are regenerated cellulose, cellulose acetate, non-woven acrylic copolymer, polysulphone, polyether sulphone, polyacrylonitrile, polyamide and the like. The biologically active material is immobilized in the pores and/or on the surface of the side of said membrane that faces away from said whole blood. Alternatively said biologically active material may be bound to an unsoluble matrix behind the membrane. Thereby the blood corpuscles are prevented from contacting said active material.

After the passage through the treating unit said whole

blood is reinserted in the patient.

Through the above construction of the microporous membrane, i.e. asymmetric immobilizing of said biologically active material, said whole blood need not be exposed to any following filtering for removing possible remaining harmful residues. As well the separation as the removal of said substances can thereby be performed in one and the same step.

CLAIMS

1. A process for the treating of and/or removal of substances from a liquid, especially whole blood, via a microporous semipermeable membrane (13, 14, 15; 19; 21, 21'; 22; 24) through adsorption and/or biological reactions by means of a biologically active material, characterized in that a penetrating fraction of said liquid is caused to flow in an alternating flow path through said microporous membrane (13, 14, 15; 19; 21, 21'; 22; 24) in each direction for contacting said biologically active material.

2. A process in accordance with claim 1, characterized in that said biologically active material is chosen among biologically active proteinaceous materials.

3. A process in accordance with claim 1, characterized in that said biologically active material is chosen among antibodies, antigens, enzymes, protein A etc., for example killed bacteria or fragments thereof.

4. A process in accordance with claim 1, 2 or 3, characterized in that said liquid is exposed to pressure variations of the order of magnitude of from -200 to +200 mmHg, preferably from -100 to +100 mmHg.

5. A process in accordance with claim 4, characterized in that said pressure variations are provided with a frequency of the order of magnitude of 0.05 - 10 Hz, preferably 0.5 - 1 Hz.

6. A device for the treating of and/or removal of substances from a liquid, especially whole blood, via a microporous semipermeable membrane (13, 14, 15; 19; 21, 21'; 22; 24) through adsorption and/or biological reaction by means of a biologically active material, characterized in that said device (3) comprises means for providing of an alternating flow of a penetrating fraction of said liquid through said membrane (13, 14, 15; 19; 21, 21'; 22; 24) in each direction.

7. A device in accordance with claim 6, characterized in that said biologically active material is immobilized in the pores and/or on the surface of the side of said membrane (13, 14, 15; 19; 21, 21') that faces away from said liquid.

8. A device in accordance with claim 7, characterized in that said biologically active material is chosen among biologically active proteinaceous materials.

9. A device in accordance with claim 8, characterized in that said biologically active material is chosen among antibodies, antigens, enzymes, protein A etc., for example killed bacteria and fragments thereof.

10. A device in accordance with one or more of claims 6-9, characterized in that said microporous semipermeable membrane (13, 14, 15; 19; 21, 21'; 22; 24) consists essentially of one or more of the following materials: regenerated cellulose, cellulose acetate, non-woven acrylic copolymer, polysulphone, polyether sulphone, polyacrylonitrile, polyamide and the like.

11. A device in accordance with claim 10, characterized in that said membrane has pores of the order of magnitude of 0.01 - 0.8 microns, preferably 0.15 - 0.45 microns.

12. A device in accordance with any one of claims 6-11, characterized in that said membrane (22) is composed of two mechanically essentially identic membrane halves (22a, 22b), wherein said biologically active material is immobilized in the pores and/or on the surface of both sides of the membrane half (22a) that faces away from said liquid.

13. A device in accordance with claim 12, characterized in that said membrane halves (22a, 22b) are provided in an abutting relationship to each other.

14. A device in accordance with any one of claims 6-13, characterized in that said biologically active material is immobilized through covalent binding to said membrane (13, 14, 15; 19; 21, 21'; 22; 24).

15. A device in accordance with any of claims 6-14, characterized in that said means (14) for providing of an alternating flow is adapted to expose said liquid to pressure variations of the order of magnitude of from -200 to +200 mmHg, preferably from -100 to +100 mmHg.

16. A device in accordance with claim 15, characterized in that said means (4) are adapted to provide pressure variations with a frequency of the order of magnitude of 0.05 - 10 Hz, preferably 0.5 - 1 Hz.

17. Microporous semipermeable membrane (13, 14, 15; 19; 21, 21'; 22; 24) for the treating of and/or removal of substances from a liquid, especially whole blood, through adsorption and/or biological reaction by means of a biologically active material, characterized in that said biologically active material is immobilized in the pores and/or on the surface of one side of said membrane (13, 14, 15; 19; 21, 21') that faces away from said liquid.

18. Membrane in accordance with claim 17, characterized in that said biologically active material is chosen among biologically active proteinaceous materials.

19. Membrane in accordance with claim 18, characterized in that said biologically active material is chosen among antibodies, antigens, enzymes, protein A etc., for example killed bacteria and fragments thereof.

20. Membrane in accordance with claim 17, 18 or 19, characterized in that it consists essentially of one or more of the following materials: regenerated cellulose, cellulose acetate, non-woven acrylic copolymer, polysulphone, polyether sulphone, polyacrylonitrile, polyamide and the like.

21. Membrane in accordance with any one of claims 17-20, characterized in that it has pores of the order of magnitude of 0.01 - 0.8 microns, preferably 0.15 - 0.45 microns.

22. Membrane in accordance with any one of claims 17-21, characterized in that it is composed of two mechanically essentially identic membrane halves (22a, 22b), wherein said biologically active material is immobilized in the pores and/or on the surface of both sides of the membrane

half (22a) that is adapted to face away from said liquid.

23. Membrane in accordance with claim 22, characterized in that said membrane halves (22a, 22b) are provided in an abutting relationship to each other.

24. Membrane in accordance with any one of claims 17-23, characterized in that said biologically active material is immobilized through covalent binding to said membrane.

25. A process for immobilizing of IgG on and in a microporous semipermeable membrane of amine substituted polyamide via glutardialdehyde, characterized in that one side of said membrane is treated with a solution of glutardialdehyde in phosphate buffer; that the so treated membrane is rinsed with distilled water and then sucked relatively dry; that IgG, dissolved in phosphate buffer, is poured over the membrane in order to cover same; that the system is evacuated; that the coupling is allowed to proceed; and in that said membrane finally is carefully rinsed with water.

26. A process for immobilizing protein A on a cellulose membrane, characterized in that one side of said membrane is treated with chloroacetic acid for coupling of carboxymethyl groups to said membrane; that the membrane is dried and then transferred into a degassed phosphate buffer solution containing 1-ethyl-3(3-dimethylaminopropyl)carbodiimide; that the reaction is allowed to proceed, whereafter said membrane is washed with phosphate buffer for removing of excess of 1-ethyl-3(3-dimethylaminopropyl)carbodiimide; that protein A is provided to cover said membrane which then is allowed to react with said protein; and in that the membrane finally is washed with water.

Fig.1

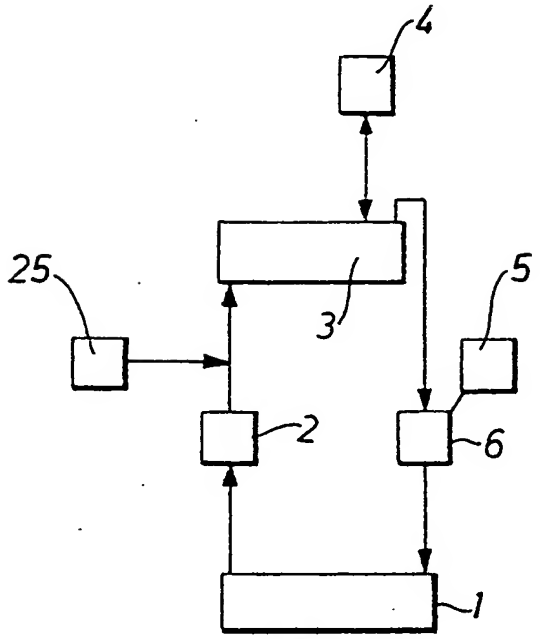


Fig.2

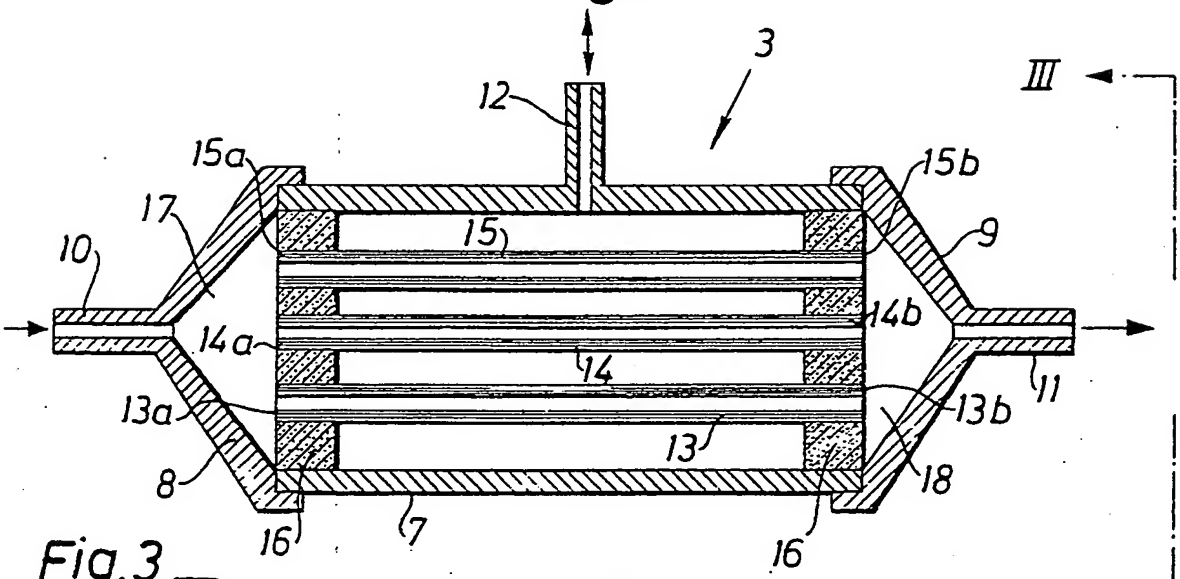


Fig.3

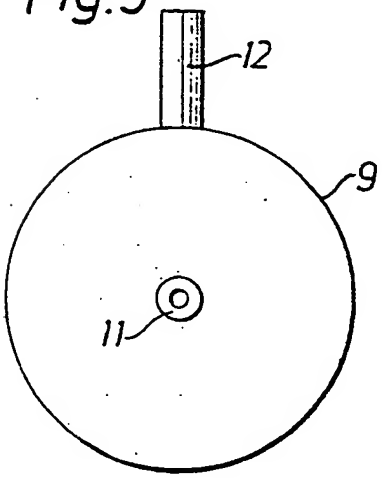


Fig.4

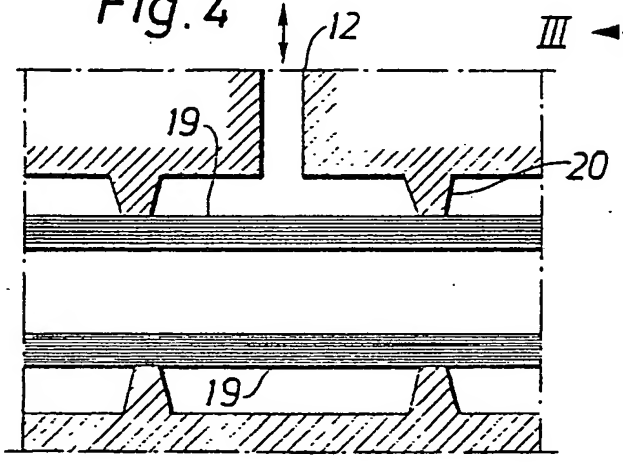


Fig. 5

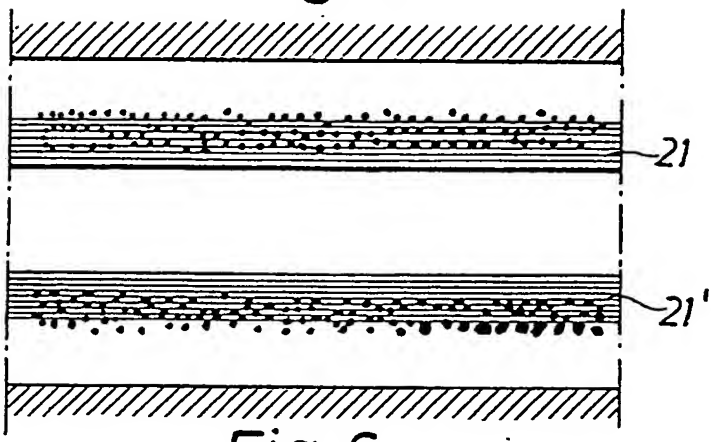


Fig. 6

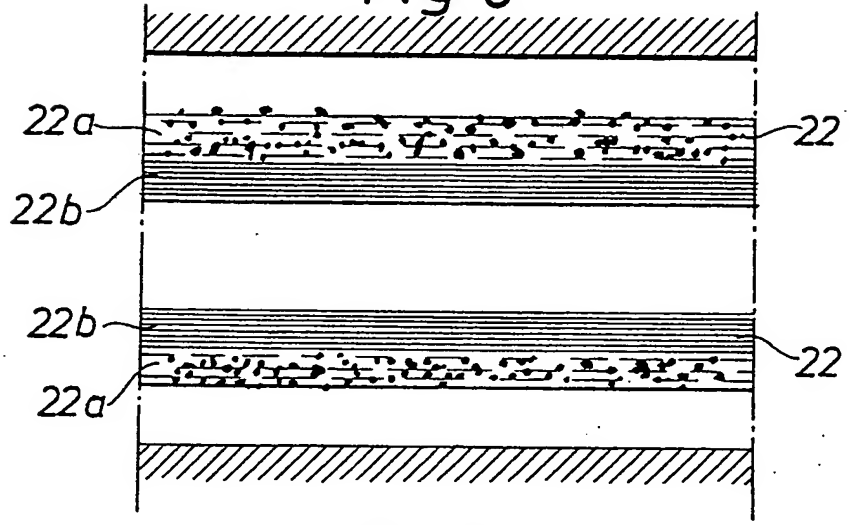
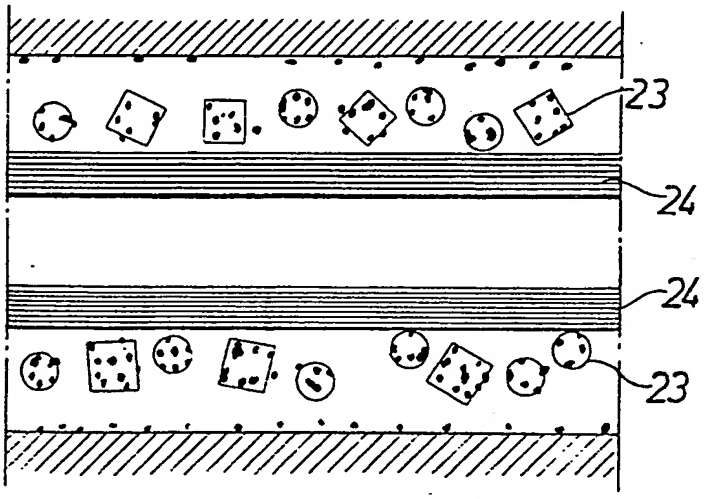


Fig. 7



INTERNATIONAL SEARCH REPORT

International Application No PCT/SE79/00132

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC [2]

B 01 D 13/00; A 61 M 1/03

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
IPC[2]	A 61 M 1/03; B 01 D 13/00; B 01 D 15/00
US Cl	210-321, 500

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	FR, A, 2 270 895 published 1975, December 12, The Community Blood Council of Greater New York Inc	1-24
X	D Thomas et al., Methods Enzymol 1976, vol 44, 901-7	1-24
A	SE, B, 373 752 published 1975, January 6, V H Hydén	
A	SE, B, 383 253 published 1972, June 12, V H Hydén et al., see especially p 3, l 19-28	3,9,19
X	FR, A, 2 404 439 published 1979, April 27, I Kato	4
X	C R Robertsson et al., Separation and Purification Methods, 5 (2), 301-332 especially 303-7 and 329 (1976)	1-24
X	DE, A, 2 225 862 published 1973, December 6, G E Henning	1-24
X	DE, A, 2 558 363 published 1977, July 7, A Affonso and H Bayer	1-24

* Special categories of cited documents: [15]

"A" document defining the general state of the art

"E" earlier document but published on or after the international filing date

"L" document cited for special reason other than those referred to in the other categories

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but on or after the priority date claimed

"T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention

"X" document of particular relevance

IV. CERTIFICATION

Date of the Actual Completion of the International Search [1]	Date of Mailing of this International Search Report [1]
1980-01-15	1980-01-30
International Searching Authority [1]	**Signature of Authorized Officer [20]**
Swedish Patent Office	Johan Auby

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

1–24

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.